J. Malac. Soc. Aust. 13: 3-14 1992

Aesthete channel morphology in three species of Australian chitons (Mollusca: Polyplacophora)

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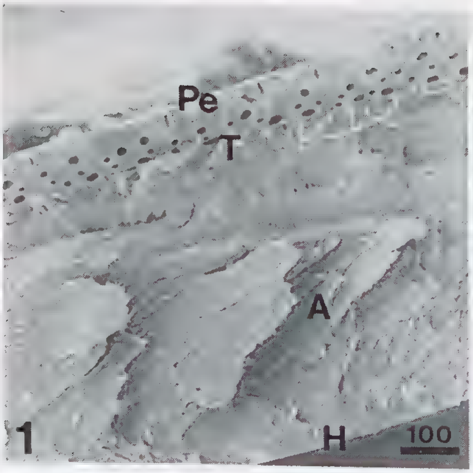
ABSTRACT

Light and scanning electron microscopy are used to investigate the internal structure of the shell valves of *Ischnochiton (Ischnoradsia) australis, Acanthopleura gaimardi* and *Cryptoplax mystica.* The shell in all species is comprised of four layers (periostracum, tegmentum, articulamentum, and hypostracum), and is perforated in specific areas by one of three classes of megalaesthete (jugal, slit-ray, and multiple branch). All classes consists of a number of micraesthete channels emanating from the distal region of a single aesthete channel, but differ according to the extent of canal branching. Both aesthete and micraesthete structures terminate at the shell surface in conical caps, while ventrally, a narrow canal extends towards the mantle interface. The gross structural arrangement is probably typical of all chiton species. A possible differentiation of aesthete function between the three types of megalaesthete is discounted in respect of ultrastructural similarities.

INTRODUCTION

Early studies of shell valve structure in chitons have been limited to the histochemistry of the periostracum and light microscopy (Knorre, 1925; Bergenhayn, 1930; Beedham and Trueman 1967). More recent investigations have employed scanning electron microscopy (Boyle, 1976; Fischer and Renner, 1979, Fischer, 1988; Currie, 1989) to examine sculpturing of the shell valve surfaces, but with the exception of Baxter and Jones (1981, 1984) the association of aesthetes with the valve layers has received little attention.

All chitons examined to date possess some form of aesthete structure, yet the functional significance of this ubiquitous organ is unresolved. The paucity of information on aesthete morphology and distribution in a range of chiton species/families, and indeed habitat, clearly remains a hindrance to our understanding of aesthete function and evolution. To this end, the present study provides comparative observations on the arrangement of aesthete ducts found within the shell valve of three chiton species from quite different habitats, representing three families in two suborders of the Polyplacophora (Kass and Van Belle, 1980): *Ischnochiton (Ischnoradsia) australis* (Sowerby, 1840) (Ischnochitonidae); *Cryptoplax mystica* Iredale & Hull, 1925 (Cryptoplacidae); and *Acanthopleura gaimardi* (De Blainville, 1825) (Chitonidae). All species are common inhabitants of the intertidal zone on the New South Wales coast, the two former species being largely confined to the bottom of the littoral zone in sheltered areas, and the latter being restricted to open surfaces on exposed headlands.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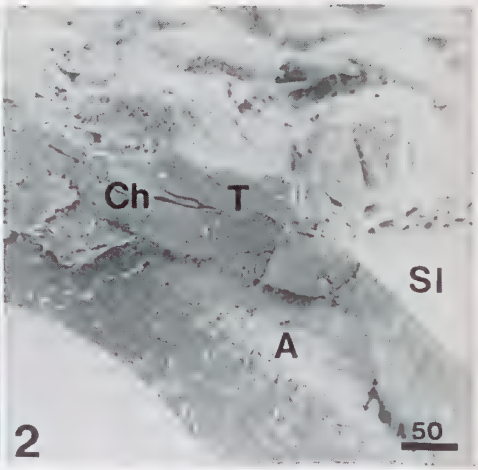
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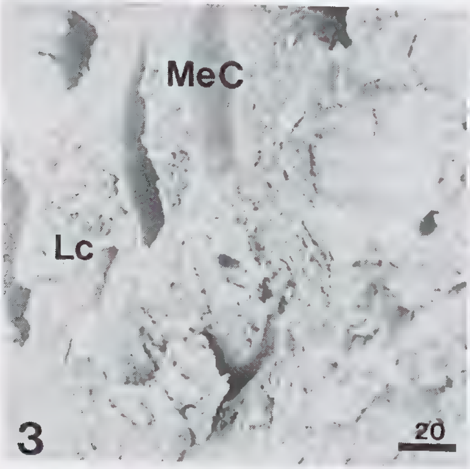
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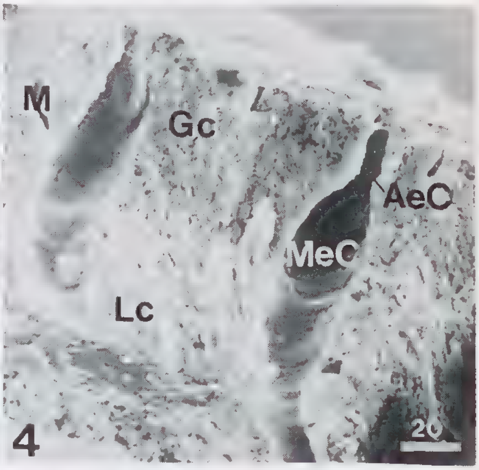
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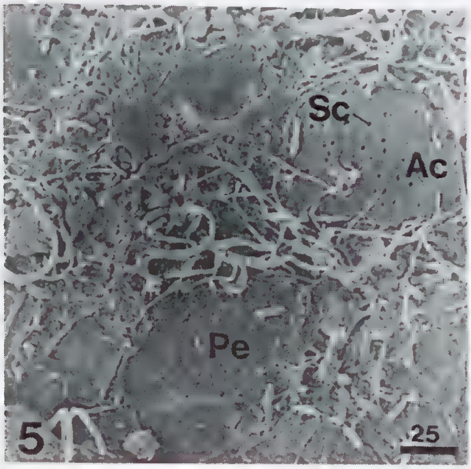
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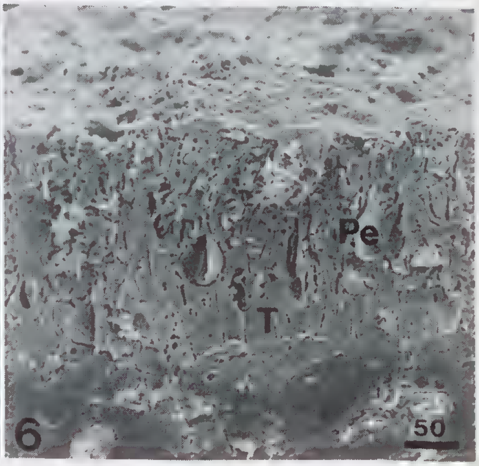
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MATERIALS AND METHODS

Animals were collected from rocks in the intertidal region at Arrawarra (30° 10′S, 153° 20′E), on the mid-north coast of New South Wales. Shell valves were dissected from specimens narcotized in 10% $MgCl_2.6H_2O$ in sea water, before being fixed in 7% formaldehyde solution in sea-water. Material to be used for light microscopy was washed in distilled water and then treated with a 5% solution of disodium ethylenediaminetetraacetic acid (EDTA) for a minimum of 48 hours or until decalcification of the valves was complete. Whole valves or sections of the decalcified material were then washed in distiled water prior to being mounted on glass slides and examined using phase contrast on a Kyowa microscope. In addition, decalcified valves were infiltrated and embedded in Paraplast wax before being transversely sectioned at 9μm and mounted on glass slides. These slides were stained in Masson's triple stain before being examined on a Kyowa microscope using bright-field illumination.

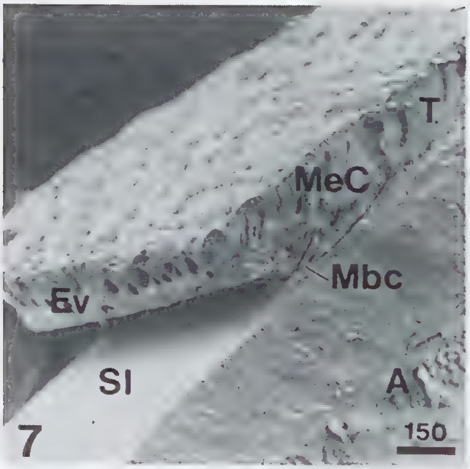
The internal arrangement of aesthete channels and their relationship to the shell valve layers was investigated by examination of surfaces exposed by fractures. After washing in distilled water the valves were ultrasonically cleaned and air-dried or dehydrated in an alcohol series. A number of valves were, in addition, treated with a 5% solution of KOH for 24 hours to remove surface organic material (Baxter and Jones, 1984). Pieces of valve were subsequently mounted with colloidal copper on aluminium stubs, sputter-coated with gold, and the fractured surfaces examined at an accelerating voltage of 20 kV on a JEOL JSM35 scanning electron microscope.

The valve nomenclature used in this study is that defined by Baxter and Jones (1984).

RESULTS

Examination of the valves of all three chiton species reveals a common structural organization comprising four separate shell layers (Fig. 1). The lowest layer, the hypostracum, is nacreous-like and consists of a thin layer of tabular crystals

◀ **Figures 1-6.** 1. Transverse section of an intermediate valve of *I. (I.) australis* showing four separate shell layers; periostracum (Pe), tegmentum (T) penetrated by numerous aesthete channels, articulamentum (A), and hypostracum (H). 2. Transverse fracture of an intermediate valve of *C. mystica* showing the articulamentum (A) extending below the tegmentum interface (T), just ventral to the main channels (Ch), to form a sutural lamina (Sl). 3. Lower region of the tegmentum exposed by a transverse fracture showing exposed megalaesthete chambers (MeC), and the arrangement of rod-like elements that comprise the laminar crystal structures (Lc). 4. Upper region of the tegmentum exposed by a transverse fracture showing two megalaesthete chambers (MeC) with associated aesthete (AeC) and micraesthete (M) channels passing towards the shell surface. Note the variation in tegmentum construction, with granular crystals (Gc) occurring below the shell surface and dorsal to the lamellar crystal structures (Lc). 5. Dorsal view of a shell valve fouled with diatoms and filamentous algae, showing the periostracum layer (Pe) which covers the entire dorsal sculpture where uneroded. Apical (Ac) and subsidiary caps (Sc) are normally visible through the periostracum. 6. Transverse fracture of a damaged intermediate valve of *A. gaimardi* showing a periostracum-like material (Pe) within the exposed aesthete cavities which penetrate the tegmentum (T). Scale bars in micrometers.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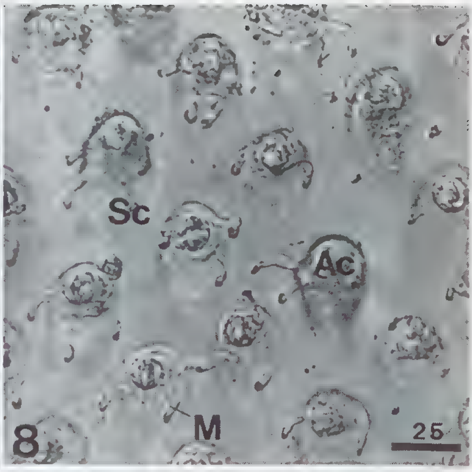
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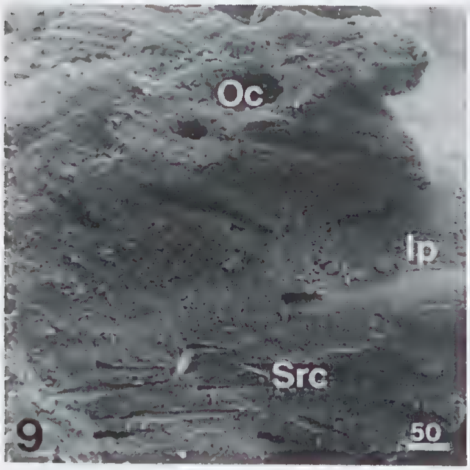
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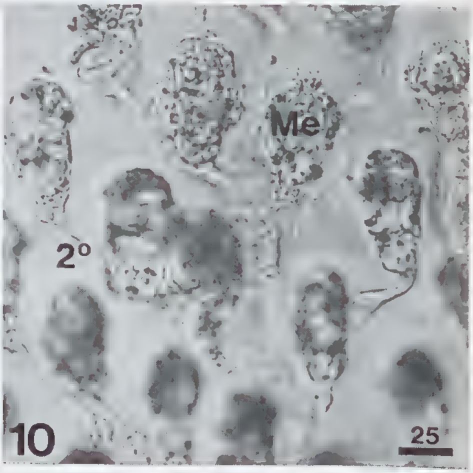
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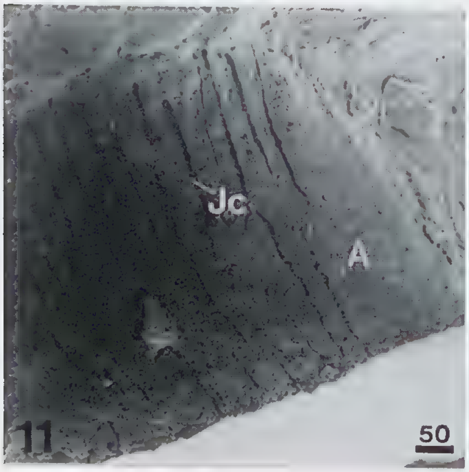
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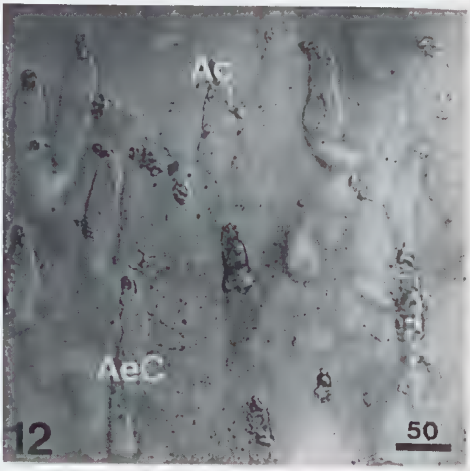
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arranged in laminae. This layer covers the ventral surface of all valves and extends dorsally to overlay the insertion plates and sutural laminae. In *C. mystica* the lamellar structure of the hypostracum is interrupted in the posterior lateral fields of the ventral surface by toriod crystals (Currie, 1989). The overlaying layer or articulamentum consists of tablet-shaped crystals arranged in a cross lamellar configuration. Lateral projections of the articulamentum (insertion plates) serve to maintain the valves in the surrounding girdle. The insertion plates on all eight valves of *I. (I.) australis*, and the six intermediate valves of *A. gaimardi*, are divided by slit rays of radiating pores in the articulamentum which emanate ventrally from the apices of the valves and terminate at the margins of the insertion plates. *C. mystica* possess no such slit rays although the articulamentum of valves II-VIII is perforated throughout the jugal area. Anteriorly the articulamentum of the second and subsequent valves, in all three species, shows two further projections or sutural laminae. These structures extend beyond the anterior border of the other major shell valve layer, the tegmentum (Fig. 2), and underlie the next anterior valve serving to anchor the valves together. The tegmentum is the dorsally visible part of the shell which exhibits an ornate sculpture of ridges and raised papillae. Like the articulamentum, the tegmentum is composed of numerous parallel rod-like elements which are arranged in blocks and aligned in a cross lamellar fashion (Fig. 3). Dorsally the rod-like elements are replaced by aggregates of small fine-grained structures (Fig. 4). Aesthete channels of varying dimensions pass obliquely through the tegmentum. These channels originate below small (4 to 14 μm diameter) conical structures termed apical and subsidiary caps (see Currie, 1989 for a full account of cap distributions on the valves of all three species examined in this study). The channels may extend laterally through the tegmentum terminating as perforations in the eave tissue, or at specific sites may pass ventrally through the articulamentum to terminate as slit rays or jugal openings. A thin organic membrane, the periostracum or fourth shell layer, covers the entire dorsal surface of the tegmentum (Fig. 5). This layer is often absent from the valve surface of older animals due to natural erosion or weathering of the shell surface, although in some

◀ **Figures 7-12.** 7. Shell fracture showing the main channel of a multiple branch aesthete channel (Mbc) extending from the lower region of the eave tissue (Ev), adjacent to the sutural lammina (Sl), and passing horizontally through the tegmentum (T) just dorsal to the articulamentum (A). Megalaesthete cavities (MeC) are produced at regular intervals along the entire length of the main channel. **8.** Dorsal view of a decalcified valve showing aesthete and micraesthete channels (M) extending upwards from the megalaesthetes to terminate at the shell surface in apical (Ap) and subsidiary caps (Sc). **9.** Transverse fracture between the insertion plates (Ip) of an intermediate valve showing the lower regions of slit ray channels (Src) passing obliquely upwards from the holes which penetrate the ventral surface. Note also an ocellus cavity (Oc) on the dorsal surface. **10.** Dorsal view of a decalcified valve showing secondary branches (2°) from slit ray channels forming rows of megalaesthete structures (Me) just below the shell surface. **11.** Transverse fracture in the jugal region of a intermediate valve showing unbranched jugal channels (Jc) passing vertically from the ventral surface through the articulamentum (A). **12.** Dorsal view of a decalcified valve showing unbranched aesthete channels (AeC) passing upwards through the shell layers and terminating at the valve surface in apical caps (Ac). Scale bars in micrometers.

valves where the tegmentum has been seriously damaged, a periostracum-like material has been found lining the aesthete channels (Fig. 6).

The aesthete channels which penetrate the various shell valve layers of *Lepidochitona cinereus* (L.) have been classified as multiple branch, slit ray, and jugal, according to the point of entry into the valve and the extent of branching of the channel (Baxter and Jones, 1981). For comparitive reasons the Baxter and Jones classification system is reiterated here:

Multiple branch channels originate from the lower rows of holes perforating the eave tissue (Fig. 7). The channels pass horizontally through the tegmentum and branch repeatedly at regular intervals. The branches, in turn, pass diagonally upwards to form bulb-shaped megalaesthete cavities, which terminate at the shell surface in apical caps (Fig. 8). Micraesthete channels are produced along the length and circumference of the megalaesthete cavities. These short ducts radiate towards the shell surface and terminate at subsidiary caps.

Slit ray channels emanate from the rows of slit ray holes which perforate the ventral surface, and in addition, from the holes in the eave tissue between the slits of the insertion plates (Fig. 9). These channels pass obliquely upwards, away from the valve margin, and in line with the slit rays. Each channel gives rise to two lateral branches in the tegmentum, upon which up to four separate secondary branches may be produced, each developing to form a single megalaesthete complex (Fig. 10).

Jugal area channels originate from the holes which perforate the ventral surface of the jugal area of the intermediate and tail valves. Each hole in the hypostracum gives rise to a single unbranched aesthete channel (Fig. 11), which passes vertically through the shell layers to form a single megalaesthete chamber in the tegmentum (Fig. 12).

The distribution of aesthete channels on the head, intermediate, and tail valves of all three species is summarized in Fig. 13, and qualified below:

Ischnochiton (I.) australis

Multiple branch channels are found in all valves of *I. (I.) australis*. These consist of long primary ducts which pass horizontally through the tegmentum and give rise to upwardly directed branches at approximately 130μm intervals. Primary channels originating from holes in the anterior edge of the valve pass through the tegmentum in a posterior direction, whereas those from holes on the lateral edges pass diagonally towards the apex of the valves. In the posterior valve, channels emanating from the lateral and posterior edges pass towards the mucro.

Slit ray channels intersect much of the valve area comprising multiple branch channels. These slit ray channels produces up to eight megalaesthete structures, arranged at the shell surface as parallel rows of apical caps.

Each of the numerous holes which perforate the triangular region of the ventral surface between the sutural lamina and the apex (in all intermediate valves), or mucro (in posterior valves), represents the entry point a single unbranched aesthete channel. This is confirmed by comparison of the ventral jugal hole density on intermediate valves (312 holes/mm^2), with the dorsal jugal apical cap density (288 caps/mm^2).

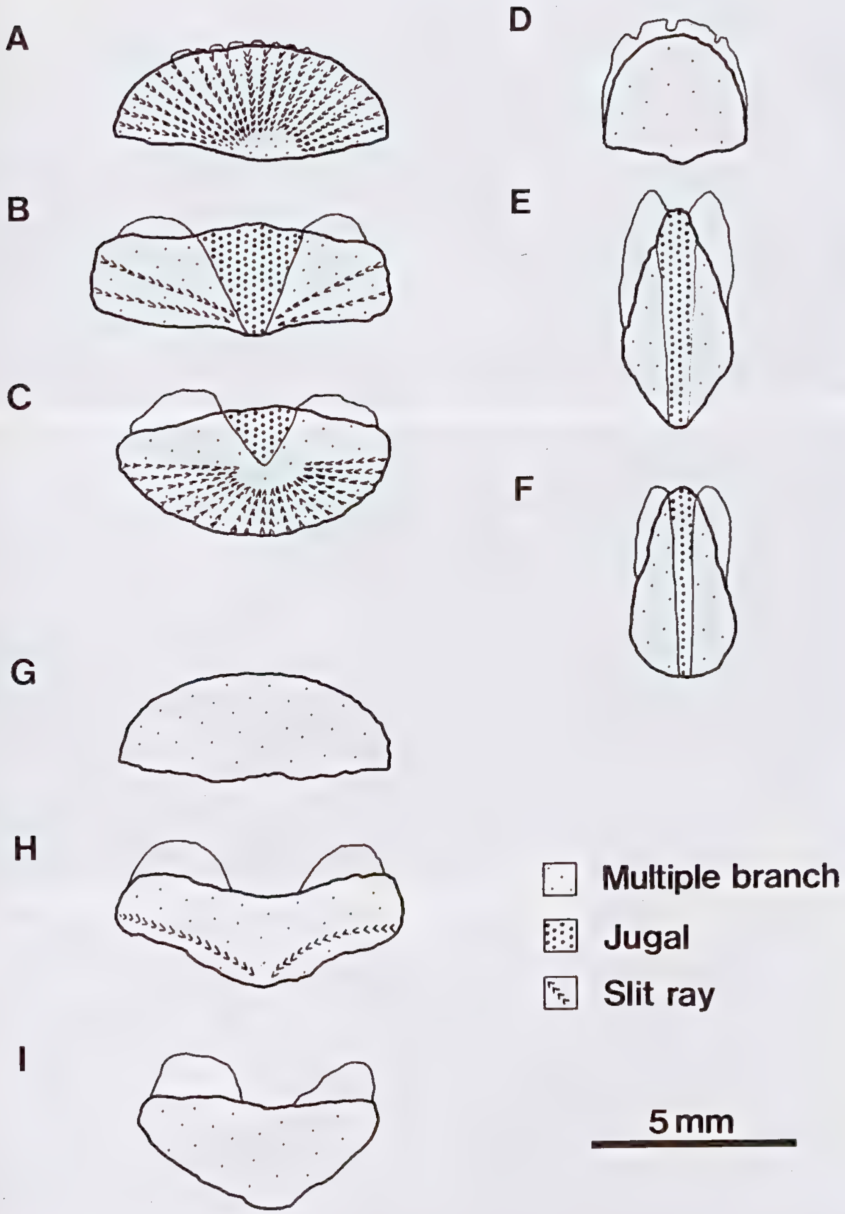
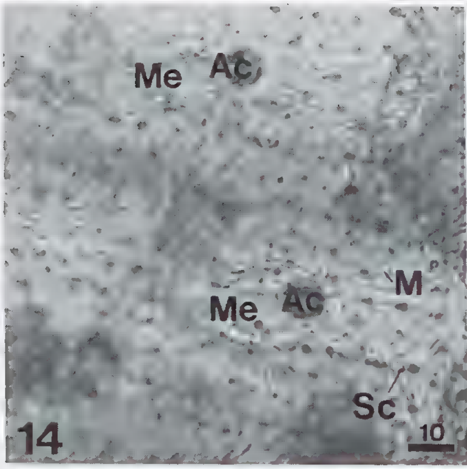


Figure 13. Schematic diagram representing the distribution of the three categories of aesthete channel on the head, intermediate, and posterior valves of *I. (I.) australis* (A-C), *A. gaimardi* (D-F), and *C. mystica* (G-I).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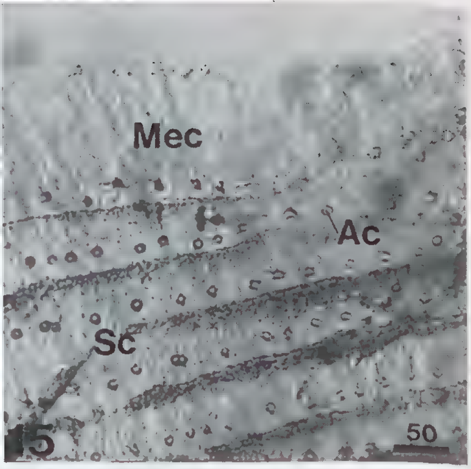
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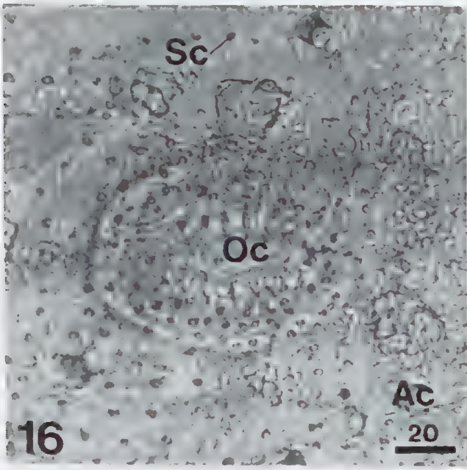
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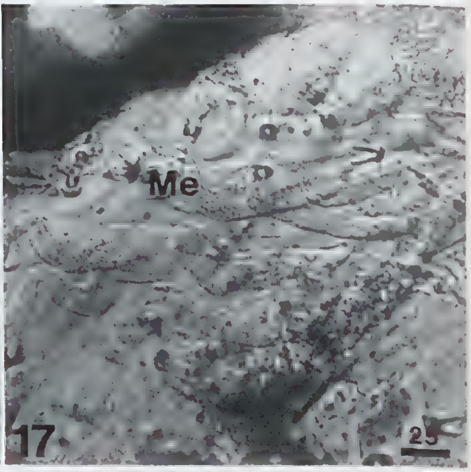
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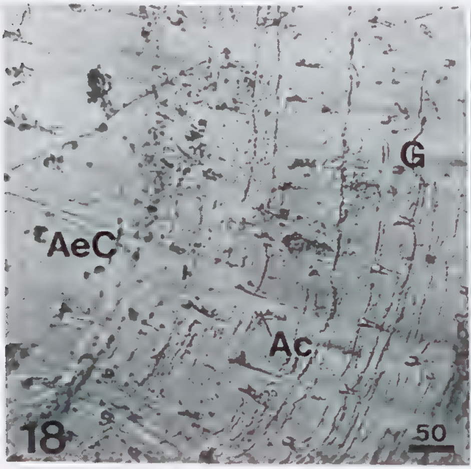
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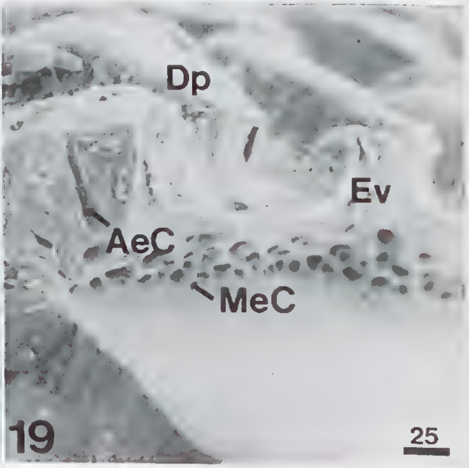
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Acanthopleura gaimardi

Multiple branch aesthete systems penetrate the majority of the tegmentum area in all valves of *A. gaimardi*, including the jugal regions. These channels originate from holes which perforate the eave tissue throughout the anterior and lateral margins of valves I-VII, and the entire margin of the posterior valve. Each canal passes horizontally through the tegmentum to converge at the apices of the anterior and intermediate valves, or the mucro of the posterior valve. Secondary branches come off at intervals of approximately 130μm, each passing directly upwards, and forming a megalaesthete with associated apical and subsidiary structures (Fig. 14). Such is the regularity of branching along the primary ducts of the multiple branch channels which penetrate the jugal region, that the apical caps on the adjacent shell valve surface are arranged in parallel lateral rows (Fig. 15).

Ocelli structures (Fig. 16) are randomly distributed throughout the frontal and lateral regions of valves I-VII, and along the posterior edge of valve VIII (Currie, 1989). A single duct leads from the base of each ocellus, and passes obliquely through the tegmentum to the eave tissue. Aesthete channels were not observed to merge directly with the ocelli structure, although proximal micraesthete channels often make contact with peripheral retinal cells situated below the ocelli lens.

Slit ray channels are present on intermediate valves only. Here, each hole in the hypostracum gives rise to a single aesthete channel, which branches once in the tegmentum to form a pair of megalaesthetes.

Cryptoplax mystica

The multiple branch aesthete channels which penetrate the tegmentum on all valves of *C. mystica*, produce secondary branches at approximately 180μm intervals, each of which give rise to a single megalaesthete complex (Fig. 17). In valve I, all primary channels pass horizontally from their point of entry on the anterior or lateral eaves to converge near the central posterior edge of the valve. The primary channels which perforate the lateral regions of valves II-VIII, are

◀ **Figures 14-19.** **14.** Dorsal view of the multiple branch region of a decalcified valve of *A. gaimardi* showing each megalaesthete (Me) giving rise to a single aesthete channel and cap (Ac) as well as numerous micraesthete channels (M) and associated subsidiary caps (Sc). **15.** Dorsal view of the jugal region of a decalcified valve of *A. gaimardi* showing megalaesthete channels (Mec) passing posteriorly through the tegmentum from the anterior shell edge. Each channel in this region branches at regular intervals to form rows of megalaesthetes with associated apical (Ap) and subsidiary caps (Sc). **16.** Dorsal view of an ocellus (Oc) on a decalcified valve of *A. gaimardi*. Apical (Ac) and subsidiary caps (Sc) are arranged about the circumference of the ocellus lens. **17.** Dorsal view of the lateral region of a decalcified, intermediate valve of *C. mystica* showing rows of megalaesthetes (Me) comprising a single multiple branch channel. **18.** Dorsal view of the jugal region of a decalcified valve of *C. mystica* showing unbranched aesthete channels (AeC) passing through the shell layers, and terminating at the shell surface in single apical caps (Ac). Growth check lines (G) are clearly visible in this region. **19.** Lateral view of the eave tissue margin (Ev) showing a new aesthete cavity (AeC) forming in association with a dorsal papillae (Dp). This cavity will eventually merge with one of the megalaesthete cavities (MeC) ventral to it on the eave tissue, and form a multiple branch aesthete system. Scale bars in micrometers.

arranged in posteriorly directed parallel lines, approximately 80μm apart. As a result the central regions of anterior valves display a higher density of apical caps than the associated lateral regions of intermediate and posterior valves (Currie, 1989).

Unbranched channels penetrate the narrow strip of unsculptured shell that runs anterio-posteriorly throughout the dorsal surface of valves II-VIII (Fig. 18). These jugal channels vary only slightly in diameter (from approximately 20μm diam. in articulamentum, to 30μm diam. in tegmentum), as they pass dorsally through the shell layers in an approximately straight line.

Decalcified valves of *C. mystica* often display an irregular distribution of pigmented material in the tegmentum. Valve fractures reveal this pigment to be enclosed in chambers of varying size and shape. These chambers do not open to the shell surface, but may form connections with megalesthete channels opening at the eave tissue.

Valve growth

Shell growth in all three species occurs along the anterior and lateral edges of valves I-VII, and throughout the entire margin of valve VIII. By examination of the variation in channel construction along the eave tissue margins and exposed surface fractures (Fig. 19), it is possible to deduce the stages of development involved in the formation of new aesthete structures with shell growth. Observations are constant with the description provided by Baxter and Jones (1984) for *Callochiton achatinus* (Brown, 1827). The sequence of aesthete construction occurs in unison in adjacent multiple branch aesthetes in *A. gaimardi*, and results in parallel rows of megalaesthetes. In *I. (I.) australis* and *C. mystica* adjacent multiple branch systems are out of phase due to the alternation of papillae on the dorsal surface.

DISCUSSION

The basic four layer structure of the shell valves examined in this study is consistent with that of other polyplacophorans (Fischer-Piette and Franc, 1960; Boyle, 1972; Baxter and Jones, 1981, 1984).

The periostracum, a thin, pliable, fibrous material constitutes the uppermost layer. It covers the entire dorsal surface of all valves except for those areas overlaid by the surrounding girdle. As in other molluscs, the periostracum is secreted by the mantle at the shell plate margin (Haas, 1972), and is subsequently hardened by a quinone tanning process (Saleuddin and Petit, 1983). The functional significance of this layer is unclear; possibly it protects the shell from erosion, and/or serves as a matrix for the deposition of new shell material. Periostracum does, to some extent, protect the shell from acid dissolution, although its chemical durability in the field is of questionable importance. Moreover, periostracum is often absent from the valves of older individuals. Quinone tanned periostracum has been postulated to be an essential prerequisite for calcification (Beedham and Trueman, 1968; Taylor and Kennedy, 1969; Chan and Saleuddin, 1974; Carter and Aller, 1975; Petit et al., 1980b), providing a suitable substratum for the orderly deposition of calcium carbonate. However, a visual examination of the principal growth regions (the eave tissue) on the valves of the three chiton species in this study, failed to reveal a layer of periostracum.

The two major shell layers, the tegmentum and articulamentum, exhibit a characteristic cross lamellar structure; consisting of thin blocks comprised of parallel rod-like crystals, arranged such that the crystals of each adjacent block lay obliquely. This alternation in angle of adjacent blocks (lamels) is thought to contribute strength and solidity to the valves of chitons (Bøggild, 1930). The processes involved in the formation of cross lamellar shell remains unresolved. Nakahara et al. (1981) described the formation of cross lamellar shell in *Strombus gigas* (L.) as beginning with fine granular particles that appear to develop into single layered envelopes (presumably empty, packet-like structures). Rod shaped crystals then form within the 'envelopes'. This may explain the granular appearance of the upper tegmentum region, although dorso-vental thickening of the shell valves is limited. It does not however explain the anomaly in shell structure along the valve margins, where the majority of shell growth occurs. Here, examination of the outer surfaces of the tegmentum and articulamentum reveals the latter to be differentially covered by an extension of the ventral shell layer (the hypostracum). Clearly two separate processes are involved in the formation of cross lamellar shell in the lateral fields: one requires the deposition of a nacreous-like layer as a precursor to, and following, the deposition of cross lamellar crystals (as in the articulamentum), the other allows the direct, lateral extension of existing lamels (as in the tegmentum).

Three distinct types of aesthete channel were found to penetrate the various shell layers of *I. (I.) australis*: multiple branch, slit ray, and jugal. The occurrence and distribution of these structures are similar to that described for the closely related *L. cinereus* (see Baxter and Jones, 1981), although slight differences in distribution are obvious when compared with another member of the Ischnochitonidae family, i.e. *C. achatinus* (see Baxter and Jones, 1984). The diversity in aesthete complexity suggests a capacity to perform a variety of functions. However, T.E.M. examination of the fine structural detail of the three aesthete classes failed to reveal any differences (Currie, in press). Considering the absence of jugal channels from the valves of *A. gaimardi*, and slit ray channels from *C. mystica* valves, it would appear that aesthete channel differentiation is of little importance in affording a range of functions, and merely represents inherent differences in shell growth associated with the formation of major valve sculptures i.e. insertion plates.

Shell valve growth was found to occur along the anterior and lateral edges of valves I-VII and throughout the entire margin of valve VIII. This observation is consistent with those of Fischer-Piette and Franc (1960) and Baxter and Jones (1981, 1984), but contradicts the findings of Beedham and Trueman (1967), who concluded that valve growth occurred along the posterior and lateral edges. Epithelial control of shell deposition in these regions presents a perplexing problem in itself, but is further complicated by the formation of aesthetes. Baxter and Jones (1981) suggest that aesthetes are derived from the cells of the mantle epithelium, and formed when directly engulfed by the adjacent growing edge. Analysis of decalcified valves shows that all aesthete structures are directly (in the case of jugal channels), or indirectly (in the case of multiple branch channels) in contact with, and quite possibly derived from, the mantle epithelium.

ACKNOWLEDGEMENTS

I would like to thank Mr P. Garlick for his valuable assistance with the S.E.M. I am also most grateful to Associate Professors Klaus Rohde and Rodney Simpson of the Zoology Department, University of New England, for their advice in preparing the manuscript. Finaly I would like to thank Dr. Alan Jones and Ms Karen Gowlett-Holmes for critically reading the final draft.

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